

# Location-specific responses to nociceptive input support the purposeful nature of motor adaptation to pain

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Title: Location specific responses to nociceptive input support the purposeful nature of motor adaptations to pain

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## INTRODUCTION:

Pain-adaptation theories propose that motor strategies change purposefully during pain, to reduce stress on irritated tissues [17,24]. Adaptation of muscles that act on the knee, can be investigated using transient acute pain elicited by hypertonic saline injection into the muscles [30] or infrapatellar fat pad [1]. Adaptations to unload these muscular or non-muscular tissues to reduce nociceptive activity might be expected to depend on the location of noxious stimulus. Consistent with this proposal, knee extension force angle changes during noxious stimulation of the medial fat pad, presumably to unload the painful tissue [32]. Likewise noxious stimulation of specific muscle regions would be expected to change muscle activation to protect the irritated muscle tissue. Yet when vasti muscle activation (electromyography; EMG) or muscle stress (shear wave elastography) have been investigated in the region of noxious input, consistent decreases have only been observed when the contralateral limb could produce compensatory force in a bilateral task [19,30], but not during unilateral, isometric knee extension when redistribution of activity between muscles/muscle regions, within a limb, would be required to unload the painful part [19,30]. Failure to observe such changes might be explained by the limitations of single-channel EMG, which assess net changes in a broad muscle area, precluding observation of changes in individual units/small areas.

The complex anatomy of the quadriceps muscle [2] and its potential to produce forces in different directions [22] makes interpretation of changes in muscle activation difficult when measured from a single region. Further, as motoneurons innervate muscle fibers that are spatially localized to regions within the vastus medialis (VM) [11,12] and rectus femoris [3], variation of motor unit recruitment between regions within each quadriceps muscle may be a strategy to adapt to pain. Potential for regional variation of vasti muscle activity with intramuscular saline injection has been observed when measured with intramuscular electrodes in 8 locations within VM and vastus lateralis

(VL) [33]. However, the spatial interpretation of this finding is unclear as the location of the intramuscular electrode within the motor unit territory is unknown. High-density surface electromyography (HDsEMG) has revealed regional re-distribution of activation within other large, multifunctional muscles such as the trapezius [7,23] and masseter [4]. This technique provides a powerful tool to assess regional variation of muscle activation in response to experimental pain. As some data also show persistence of modified motor unit recruitment strategy after pain resolution [31] HDsEMG could identify whether this maintains redistribution of activation between regions of the muscle.

This study aimed to determine whether; i) quadriceps muscle activation and ii) knee force direction is modulated in a manner that is specific to location of acute noxious input (experimental pain) at different locations within the quadriceps muscle and non-muscular tissue of the knee. We hypothesized that activation would be inhomogeneously reduced across regions of the quadriceps muscle and that adaptation of muscle activation and force direction would differ when experimental pain is induced in different locations. We also investigated whether adaptive motor strategies persist after pain resolution.

## **METHODS:**

### **Participants**

Fourteen individuals with no current knee pain and no history of lower limb surgery or neuromuscular disorders participated in this study (7 women; 18 - 47 years old). Participants provided written informed consent prior to the experimental session. The study conformed to the standards of the latest revision of the *Declaration of Helsinki* (2013) and was approved by the Human Research Ethics committee of the University of Queensland (2004000654).

## **Experimental Protocol**

The dominant leg (leg used to kick a ball) was tested for all participants. Sitting in a custom-built chair, participants performed isometric knee extension contractions with the hip flexed to 100° and the knee flexed to 60° from full extension. Participants were asked to keep their arms crossed over their chest during all contractions and were visually monitored to avoid visible compensations or changes in posture. The ankle was strapped to a 3-D force sensor (Sensix, France), positioned 2 cm proximal to the medial malleolus. At the beginning of the session, participants performed three maximal isometric voluntary contractions (MVC) of the knee extensor muscles for 5 s with strong verbal encouragement.

## **Electromyography and Force Recordings**

The skin was cleaned with abrasive gel (Neuprep, Weaver and Company, USA) and surface EMG signals from VM and VL were collected using two HDsEMG grids of 64 electrodes (semi-disposable adhesive matrix; OTBIOelettronica, Torino, Italy) arranged in 5 columns and 13 rows spaced by 8 mm (Fig. 1). Each grid was placed with its long axis aligned to the muscle innervation zone. The innervation zone was localized prior to electrode placement using a linear electrode array placed along the approximate muscle fibre direction (16 silver bar electrodes, 10-mm inter-electrode distance, OTBIOelettronica, Torino, Italy) that was moved over different regions of the muscle while the participants maintained a low-force isometric knee extension contraction. The medial and lateral boundaries of VM and VL were identified prior to grid placement using ultrasound imaging (Logiq e, GE Medical Systems, China) to ensure that all the electrodes were placed over the muscle of interest. The electrode grid was held in place using bi-adhesive foam. Conductive paste (Ten20, Weaver and Co., Aurora, CO, USA) facilitated good electrical contact between the skin and electrodes. Reference electrodes were placed over the patella and the medial and lateral femoral condyles. HDsEMG signals were collected in monopolar mode using a HDsEMG amplifier (128-channel EMG-USB; OTBIOelettronica,

Torino, Italy). HDsEMG signals were amplified  $\times 500$  and digitized at 2048 Hz using a 12-bit A/D converter.

As a surrogate measure for forces applied to the patella and potentially pressure on the fat pad, three-dimensional components of the isometric knee extension force were recorded using a triaxial force sensor (Sensix, France), amplified ( $\times 500$ ), filtered (band-pass 10-750 Hz) and collected using the HDsEMG amplifier. Surface EMG of the rectus femoris muscle was collected using a pair of electrodes (Ambu Blue Sensor N, Denmark – interelectrode distance: 20 mm), placed over the rectus femoris (RF) muscle belly,  $\sim 5$  cm proximal to the VM grid. Rectus femoris EMG signals were pre-amplified ( $\times 1000$ , Wave Wireless EMG, Cometa, Italy), filtered 10-500 Hz, digitized at 2000 Hz using a 1401 (CED, UK) and collected using Spike2 v7 (CED, UK). To synchronize the force and EMG recordings, the force signal was split and simultaneously digitized by the two acquisition systems.

### **Experimental knee pain**

Acute pain was induced by single bolus injections of hypertonic saline (medial infrapatellar pad: 0.25 ml, 5%; muscle: 0.5 ml, 7%), using a 1-ml syringe and 25G needle (25 mm). The order of the injections was randomized before each experiment. Injection into the fat pad was performed according to previous studies [16]. Muscle injections were performed  $\sim 5$  mm medially to row 12 (VMD), laterally to row 2 (VMP) or medially to row 4 (VL, see Fig.1). Pain intensity was assessed using a numeric rating scale (NRS) from 0 to 10, anchored with “no pain” at 0 and “worst pain imaginable” at 10. Participants rated the level of pain they experienced during each contraction immediately after the contraction was completed. Participants also marked the outline of the painful area on their leg at the end of each condition; photographs were taken and scanned to trace the painful regions. As muscle twitches and cramping were reported after saline injection during pilot testing, participants were asked to report the occurrence of any involuntary muscle activation following the injections. Eight out of 14 participants

were naïve to hypertonic saline injections. Similarly to previous studies [2,10,13] and because of the large number of injections required, we did not have a control condition where isotonic saline is injected. As isotonic saline injections were shown not to change vasti neuromuscular activation [12,14] or regional muscle activation [3,11], we did not consider necessary to include a control condition in this study.

### **Voluntary Contractions**

The experimental task consisted of five repetitions of a 10% MVC isometric knee extension ramp contraction with visual feedback (5-s ramping up, 10-s hold, 5-s ramping down, 10-s rest). Each set of 5 ramps was performed before the first injection (*baseline*), after each of four injections of hypertonic saline solution in different locations, respectively, beginning when the participant reported a pain of at least 3/10 (*pain*), and ~1 min after resolution of the pain (0/10; *post-pain*) for each of the pain locations, respectively. Prior to the baseline trial, participants performed the 5 ramps once to familiarize themselves with the task.

### **Data analysis**

Signals were imported and analyzed using Matlab (v.2016a, The MathWorks, USA). Pain ratings were averaged across the 5 ramps for each pain condition. Force signals were low-pass filtered at 10 Hz (Butterworth, 2<sup>nd</sup> order). In line with a previous study [26], changes in the direction of force were quantified as the angle between the knee extension and tangential force vectors (FX: medio-lateral; FY: proximo-distal). The average knee extension force amplitude and force direction angles were averaged across the five ramps. For each trial, the force offset (calculated 500 ms before the beginning of the first ramp) was subtracted from the average value to account for subtle changes in lower limb position between trials.

EMG signals were band-pass filtered at 10-400 Hz (Butterworth, 2<sup>nd</sup> order). Quality of the surface EMG signals was carefully assessed and HDsEMG channels that showed noise or large 50 Hz interference (due to poor skin-electrode contact) were excluded and replaced by the linear interpolation of four adjacent channels. For each ramp, a 5-s epoch between the 4<sup>th</sup> and 9<sup>th</sup> second of the 10-s hold phase was extracted and used for the analyses of both EMG and force. The intensity of muscle activation was calculated as the average rectified value (ARV) from each channel of the grid (VM and VL) and the electrode pair (RF) within the same 5-s epoch of each ramp. This resulted in two ARV amplitude spatial distributions (13x5 channels, VM and VL) and a single ARV value for RF for each condition. The overall activation level of VM and VL was quantified as the average of the 5 highest amplitude values across the electrode grid. To characterize spatial variations of the EMG distribution in the *pain* and *post-pain* conditions, distributions of normalized change scores (NCS) were calculated using the following formula for each channel of the grid:

$$NCS_i = 100 * (TRIAL_i - BAS_i) / BAS_{MEAN}$$

where i is the channel of the grid, TRIAL is the ARV distribution of VM or VL during individual *pain* and *post-pain* conditions, BAS is the ARV distribution of VM or VL at baseline (before any injections), BAS<sub>MEAN</sub> is the average across all the channels of the grid during baseline trials. To ensure that the results of the study were not influenced by the normalization procedure, the analyses were repeated normalizing the change score of each channel to the baseline value of the same channel, as opposed to the average of all channels at baseline. The statistical results were similar between the two analyses (data not reported).

Hence, NCS describes the percentage change of EMG amplitude for each channel of the grid relative to baseline. To identify any regional reduction in muscle activation across conditions, the 5 channels with the lowest NCS were identified (i.e. towards negative values); this procedure identified



channels with the largest decrease (or the smallest increase) of EMG amplitude during the *pain* or *post-pain* conditions compared to baseline. The identified channels were characterized in terms of position (barycenter of their coordinates) and intensity (average value). The proximo-distal coordinate of the barycenter, which is related to the position of the active muscle fibers within the VM [10], was used to represent regional activation within the VM and VL.

### **Statistical Analysis**

Statistical analyses were performed using SPSS v. 22 (IBM Inc., Armonk, NY, USA). Parametric or non-parametric tests were used according to the normality of the distribution of the data as determined with the Shapiro-Wilk test. When Mauchly's test identified a violation of the assumption of sphericity, a Greenhouse-Geisser correction was applied. All factors were considered within-subject. Pain scores were averaged across the 5 ramps, and compared across the four injection locations (FP, VMD, VMP, VL) using the Friedman test. Separate tests were performed for force and EMG amplitude analyses to compare *baseline* with *pain*, and *baseline* with *post-pain*. One-way ANOVAs were used to identify differences between *baseline* and pain (all four locations) and between *baseline* and post-pain (four locations) for normalized knee extension force and force direction angles; when significant, planned contrasts were used as post-hoc analyses to identify which locations significantly differed from baseline. Similarly, Friedman tests were used to test whether VM, VL and RF EMG amplitude differed between *baseline* and the four injection locations, separately for pain and post-pain; post-hoc analyses involved paired Wilcoxon tests to compare each individual location to baseline.

For VM and VL, additional analyses were undertaken to quantify EMG amplitude changes accounting for regional redistribution within the muscle. To identify whether muscle activity was consistently reduced in a specific region during pain, two-way ANOVAs were used to test the effect of location and pain condition (*pain/post-pain*) on the position of the channels with lowest NCS; post-hoc

tests identified which conditions were significantly different from 7 (i.e. the midpoint of the electrode grid); if the channels showing the largest decrease were scattered across the grid, or inconsistent across participants, their average position would be close to the middle of the grid; instead, if they were consistently clustered in the proximal or distal region, a significant difference from baseline would be observed. Bonferroni correction for multiple comparisons was applied to all post-hoc tests by multiplying the p-value by the number of tests. Statistical significance was set at  $p \leq 0.05$ .

## RESULTS:

Participants generally reported pain in the proximity of the injection site (Fig. 1). Pain intensity did not differ across locations ( $p=0.2$ ; FP:  $2.9 \pm 1.1$ ; VMD:  $3.4 \pm 1.2$ ; VMP:  $3.3 \pm 1.1$ ; VL:  $3.1 \pm 1.3$ ). Injections were performed  $21.0 \pm 5.2$  minutes apart; the tasks for the post-pain condition started  $\sim 1$  minute after pain returned to 0/10 which was  $13.8 \pm 4.8$  minutes after the participant reported a pain level of at least 3/10. Nine (7 men) of the 14 participants reported cramping or repetitive muscle twitches following the hypertonic saline injection. These were confirmed by the EMG data (Fig. 2). Involuntary muscle activation was observed following injection in VMP (5 participants), VMD (3 participants), VL (2 participants), but never after FP injection. One participant reported twitching and cramps in more than one location. Cramping and twitches started either before, during, or after the submaximal force task. Visual observation of the EMG distribution revealed that muscle fiber twitching/cramping was spatially localized in proximity of the site of injection (Fig.2). Because of cramping and twitches observed during the submaximal contractions, 7 ramps from 4 participants were excluded from the subsequent analysis.

Neither the normalized knee extension force, nor the proximal-distal force production angle differed from *baseline* during pain [extension:  $F(4,52)=1.01$ ,  $p=0.41$ , average across all conditions, pain:  $10.0 \pm 0.3\%$  MVC; proximal-distal: ( $F(4,52)=1.96$ , pain:  $p=0.11$ ,  $10.3 \pm 7.0^\circ$ ] or post-pain [extension:  $F(4,52)$ ,  $p=0.14$ ,  $10.0 \pm 0.3\%$  MVC); proximal-distal:  $F(4,52)=1.53$ ,  $p=0.21$ ,  $10.1 \pm 6.3^\circ$ ] conditions. In the medio-

lateral direction, participants produced forces angled  $14.4 \pm 4.4^\circ$  laterally from the knee extension force vector at baseline. Force was produced more medially both during pain ( $F(4,52)=3.46$ ,  $p=0.01$ ) and post-pain ( $F(4,52)=6.34$ ,  $p<0.01$ ; Fig.3). Post-hoc analysis indicated the medio-lateral force direction was  $3.5 \pm 3.2^\circ$  more medial during pain for FP ( $p=0.01$ ), but did not change significantly for any of the muscle injections ( $p>0.73$ ). After pain had resolved, force was produced more medially than at baseline for FP ( $p=0.01$ ), VMD and VMP ( $p<0.05$ ) but not after VL injection ( $p=0.24$ ).

During pain, Friedman tests revealed that surface EMG amplitude significantly decreased for VM (average of 5 recording sites with highest EMG;  $p<0.05$ ) and increased for RF (single electrode pair;  $p=0.05$ ), but no consistent changes across participants were observed for VL (average of 5 recording sites with highest EMG;  $p=0.43$ ). Although responses generally differed between individuals, a tendency for the activation of VM and VL to change in the same direction was observed (Fig. 4). After Bonferroni correction, post-hoc analysis with paired Wilcoxon tests identified only a tendency for decreased VM EMG amplitude when VMD was injected (median decrease: 10.4%; Bonferroni-adjusted  $p=0.08$ ); and no significant change for the other locations ( $p>0.76$ ). When the FP was injected, there was a tendency for RF surface EMG amplitude to increase by 12.6%, however this was not significant (Bonferroni-adjusted  $p=0.09$ ). Large variability was observed across participants such that no consistent changes were observed when the noxious stimulus was localized in the other muscle locations ( $p>0.9$ ). Surface EMG amplitude did not differ from baseline for any of the muscles tested post-pain ( $p>0.84$ ).

The 5 channels with the lowest NCS (i.e. larger decrease or smallest increase of EMG amplitude from baseline) were adjacent to each other in most trials (representative participants in Figs 5 and 6). For VM, a 2-way ANOVA identified a main effect of injection location ( $F(3,39)=2.89$ ,  $p<0.05$ ), but no difference between pain and post-pain ( $F(1,13)=1.39$ ,  $p=0.26$ ) or interaction between these factors ( $F(3,39)=0.32$ ,  $p=0.81$ ). As no interactions were observed, values during and post-pain were averaged

before post-hoc comparisons. The channels with lowest NCS were significantly more distal than the midpoint of the electrode grid when VMD was injected ( $3.1 \pm 1.2$  inter-electrode distances,  $t=9.7$ , Bonferroni-adjusted  $p < 0.001$ ) and a similar change in VMD was also observed when VL was injected ( $2.1 \pm 2.3$  inter-electrode distances,  $t=3.4$ , Bonferroni-adjusted  $p < 0.05$ ). No significant effects were observed for distribution of activation within VL ( $p > 0.11$ ) or on the medio-lateral coordinate of the location for either VM ( $p > 0.19$ ) or VL ( $p > 0.09$ ).

## **DISCUSSION:**

The results of this study show adaptations in force and muscle activation that depend on the location of noxious stimulus. Three key observations were made. First, regional modulation of the quadriceps was observed in response to acute noxious stimulation of the distal region of the vastus medialis or vastus lateralis. Second, force direction was modified after medial fat pad injections, but without consistent regional variation in muscle activation. Third, motor adaptations were maintained even after pain resolution.

The specificity of adaptation of force direction and muscle activation to different locations of the noxious stimulus suggests a purposeful adaptation to protect the painful region [17]. Early changes in force direction were consistently observed when the medial fat pad was injected, whereas changes following VM muscle injections reached statistical significance only post-pain. Although consistent across participants, the relevance of the small (on average  $< 4$  degrees) change in knee extension force direction observed in this study may be questionable. However, it should be considered that knee extension force direction was used as a surrogate measure for forces applied to the patella – even a small change in external force direction may possibly reflect a large change in patellofemoral joint forces. It is possible that the medial fat pad may be unloaded by posterior translation of the medial tibial condyle, which combined with knee extension, could underlie a more medially-directed force vector at

the ankle. The only trend for consistent change in muscle activation after injection of the fat pad was increased rectus femoris activation. Although not possible to resolve from this study, RF has a role in external rotation of the femur, which may contribute to the change in knee extension force vector. The absence of systematic changes in vasti activation with FP injections suggests that the changes in knee extension force direction may be due to changes in activation of muscles other than the vasti.

Consistent changes of VM activation were observed in response to VMD injection, which led to changes in spatial distribution and decreased amplitude. The location of the EMG channels with lowest change scores (i.e. greatest decrease or smallest increase) identifies that, regardless of whether activation of the VM muscle as a whole increased, decreased, or did not change with pain, the activation of the distal region of the muscle was relatively less than the remainder of the muscle during distal VM pain. This suggests preferential unloading/less loading of the region in which nociceptive stimulation was applied. Interestingly, similar changes in the amplitude distribution within the VM were observed when VL was injected with hypertonic saline, although to a lesser extent and less consistently across participants. This stereotypical redistribution of activation appears to be, in part, consistent with that observed when noxious stimuli were applied to different regions of the upper trapezius [6,7]. The absence of changes in spatial distribution when VMP was injected may be related to the different function of the distal (patellar stabilization) vs. proximal (knee extension) regions of the vasti, although further interpretation of this proposal is beyond the scope of the present investigation. Taken together, the results provide some support for the hypothesis that adaptation depends on the location of pain, as noxious stimulation of non-muscular (fat-pad) tissues mainly resulted in changes in force direction, whereas noxious stimulation of VMD or VL mainly resulted in regional changes in VM regional activation.

The results of this study show preferential modulation of individual quadriceps heads as well as regions within an individual head instead of uniform inhibition of the agonist muscles in the presence of

pain. Across participants, changes in VM and VL activation were generally observed in the same direction (i.e. both muscles either increased or decreased activation); however, VM activation reduced in response to noxious input of VM, more consistently than VL (fig.4). VM and VL share synaptic input [21], and their activity is usually co-modulated in response to pain [19], voluntary activation [18], and fatigue [20]. In contrast, experimental joint effusion shows that afferent input affects the quadriceps motoneurone pool inhomogeneously, as spinal excitability of VM was reduced with lower volumes of effusion and to a greater extent than VL or RF [28]. Differences in relative timing and amplitude of activation of VM and VL in association with experimental pain [16], delayed onset muscle soreness [14] and patellofemoral pain syndrome [5] provide further evidence of partly inhomogeneous effects of pain on the drive to vasti muscles. In addition, although several previous experiments found no evidence for a systematic decrease in VM EMG amplitude during isometric contractions when noxious input was applied to VM [18,19], here we demonstrated a trend for decreased activation in 11/14 participants. A possible reason for these differences when compared to previous studies is that changes were observed only in the distal VM when the hypertonic saline solution was injected in the same muscle region. Similar to Hug et al. [18], we observed no consistent changes in EMG in a more proximal (less distal) muscle region when that region was injected.

In this study, some adaptations were observed during pain and persisted after pain resolution, whereas others were only observed either during pain or after pain resolution. Our findings of altered distribution of activation within the muscle but unchanged overall activation post-pain agrees with the observation that motor unit discharge rate, but not the population of recruited motor units, returns to baseline after pain resolution [31]. The non-homogeneous time-course concurs with other studies that showed adaptation to corticospinal excitability both during and after pain resolution [27,29], but adaptation of spinal excitability only after pain resolution [25]. Although speculative, this may provide some indication of the underlying mechanisms. The immediate effect of muscle injection on distribution

of muscle activity may depend on adaptation to the descending command from the motor cortex, whereas the late impact of muscle injection on force direction may depend on a spinal mechanism. The time-course of effect of FP injection on knee force direction similarly implies cortical involvement, yet why this would differ with the mechanism for muscle injection is unclear. Although not explaining the apparent difference in time course of some changes, one possibility for the persistence of changes after pain resolution may be that pain is a motivator to adapt (presumably to protect the tissues), but resolution of pain is not necessarily a motivator to adapt back to the pre-pain state [17].

Involuntary muscle activation, in the form of localized cramp or repetitive muscle twitching, was observed after 10/42 muscle injections (9/14 participants), but never after fat pad injections. Visual analysis revealed the action potentials were highly localized in close proximity to the injection location. When single differential signals were calculated from the monopolar recordings, the innervation zone and action potential propagation could be observed in all cases (Fig.2). This strongly suggests the involuntary muscle activation is generated from the motor end-plate. The higher incidence of this phenomenon in this study than previous reports may relate to the location of the injections, which was performed in proximity of the innervation zone. Although previous research reported no changes in muscle fiber membrane properties following hypertonic saline injection (indirectly measured as action potential conduction velocity [8]), direct peripheral effect of saline on the neuromuscular junction cannot be excluded, especially when the injection is performed in proximity of the innervation zone. The highly localized involuntary activation in the HDsEMG recording supports three features of neuromuscular organization that are important for interpretation of the broader findings of this study. These are: 1) sensory input from the hypertonic saline injection is highly localized within the muscle, as implied by imaging studies [13]; 2) VM (and VL) motor units have most of their muscle fibers clustered within small regions of the muscle [11,12]; and 3) regional activation within the vasti can be observed as

changes of surface EMG amplitude distribution in the proximal-distal direction in the HDsEMG recordings [9,10].

A possible limitation of this study is that the adaptation to injections in a single location may have been influenced by the after-effect of a previous injection. However, the order of injection location was randomized and location-specific changes were observed for all extracted indices which suggests that a cumulative effect, if any, had a minor effect on the results.

Adaptation to acute experimental knee pain involved a systematic reduction of activation of the distal region of the VM, changes in muscle activation across the quadriceps heads, and modification of knee force direction (more medial during pain), observed to a different extent depending on whether the noxious stimulation was applied to specific locations within the quadriceps muscle tissue or to the fat-pad. Changes in spatial distribution of activity provide evidence of inhomogeneous distribution of drive to the motoneuron pool of VM in the presence of noxious input. Regional changes in muscle activation and changes to knee force direction persisted even after pain resolution, whereas changes in overall amplitude of muscle activation returned to baseline. These findings provide new insight on the short-term adaptation to pain.

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**CONFLICT OF INTEREST:** The authors declare no competing financial interests.



## REFERENCES:

- [1] Bennell K, Hodges P, Mellor R, Bexander C, Souvlis T. The nature of anterior knee pain following injection of hypertonic saline into the infrapatellar fat pad. *J. Orthop. Res.* 2004;22:116–21. doi:10.1016/S0736-0266(03)00162-1.
- [2] Blazeovich AJ, Gill ND, Zhou S. Intra- and intermuscular variation in human quadriceps femoris architecture assessed in vivo. *J. Anat.* 2006;209:289–310.
- [3] Buchtal F, Erminio F, Rosenfalk P. Motor Unit Territory in Different Human Muscles. *Acta Physiol. Scand.* 1959;45:72–87.
- [4] Castroflorio T, Falla D, Wang K, Svensson P, Farina D. Effect of experimental jaw-muscle pain on the spatial distribution of surface EMG activity of the human masseter muscle during tooth clenching. *J. Oral Rehabil.* 2012;39:81–92.
- [5] Cowan SM, Bennell KL, Hodges PW, Crossley KM, McConnell J. Delayed onset of electromyographic activity of vastus medialis obliquus relative to vastus lateralis in subjects with patellofemoral pain syndrome. *Arch. Phys. Med. Rehabil.* 2001;82:183–9. doi:10.1053/apmr.2001.19022.
- [6] Dideriksen JL, Holobar A, Falla D. Preferential distribution of nociceptive input to motoneurons with muscle units in the cranial portion of the upper trapezius muscle. *J. Neurophysiol.* 2016;116:611–618. doi:10.1152/jn.01117.2015.
- [7] Falla D, Arendt-Nielsen L, Farina D. The pain-induced change in relative activation of upper trapezius muscle regions is independent of the site of noxious stimulation. *Clin. Neurophysiol.* 2009;120:150–157. doi:10.1016/j.clinph.2008.10.148.
- [8] Farina D, Arendt-Nielsen L, Merletti R, Graven-Nielsen T. Effect of Experimental Muscle Pain on

- Motor Unit Firing Rate and Conduction Velocity. *J. Neurophysiol.* 2003;91:1250–1259.  
doi:10.1152/jn.00620.2003.
- [9] Gallina A, Blouin J-S, Ivanova TD, Garland SJ. Regionalization of the stretch reflex in the human vastus medialis. *J. Physiol.* 2017;0:1–11. doi:10.1113/JP274458.
  - [10] Gallina A, Ivanova TD, Garland SJ. Regional activation within the vastus medialis in stimulated and voluntary contractions. *J Appl Physiol* 2016;121:466–474. doi:10.1152/jappphysiol.00050.2016.
  - [11] Gallina A, Vieira T. Territory and fiber orientation of vastus medialis motor units: A Surface electromyography investigation. *Muscle Nerve* 2015;52:1057–1065.
  - [12] Gootzen T, Vingerhoets D, Stegeman DF. A study of motor unit structure by means of scanning EMG. *Muscle Nerve* 1992;349–357.
  - [13] Graven-Nielsen T, McArdle A, Phoenix J, Arendt-Nielsen L, Jensen TS, Jackson MJ, Edwards RHT. In vivo model of muscle pain: Quantification of intramuscular chemical, electrical, and pressure changes associated with saline-induced muscle pain in humans. *Pain* 1997;69:137–143.
  - [14] Hedayatpour N, Falla D. Delayed onset of vastii muscle activity in response to rapid postural perturbations following eccentric exercise: a mechanism that underpins knee pain after eccentric exercise? *Br. J. Sports Med.* 2013. doi:10.1136/bjsports-2012-092015.
  - [15] Hedayatpour N, Falla D, Arendt-Nielsen L, Farina D. Sensory and electromyographic mapping during delayed-onset muscle soreness. *Med. Sci. Sports Exerc.* 2008;40:326–334.
  - [16] Hodges PW, Mellor R, Crossley K, Bennell K. Pain induced by injection of hypertonic saline into the infrapatellar fat pad and effect on coordination of the quadriceps muscles. *Arthritis Rheum.* 2009;61:70–7. doi:10.1002/art.24089.
  - [17] Hodges PW, Tucker K. Moving differently in pain: A new theory to explain the adaptation to pain.

- Pain 2011;152:S90–S98. doi:10.1016/j.pain.2010.10.020.
- [18] Hug F, Hodges PW, Hoorn WVD, Tucker K. Between-muscle differences in the adaptation to experimental pain. *J. Appl. Physiol.* 2014;117:1132–1140. doi:10.1152/jappphysiol.00561.2014.
  - [19] Hug F, Hodges PW, Tucker K. Task dependency of motor adaptations to an acute noxious stimulation. *J. Neurophysiol.* 2014;111:2298–2306. doi:10.1152/jn.00911.2013.
  - [20] Kouzaki M, Shinohara M, Santos AD, Poston B, Jesunathadas M, Bobich LR, M T, Riley ZA, Baudry S, Enoka RM, Reid C, Gandevia SC, Taylor JL, Smith JL, Martin PG. The frequency of alternate muscle activity is associated with the attenuation in muscle fatigue. *J. Appl. Physiol.* 2010;715–720.
  - [21] Laine CM, Martinez-valdes E, Falla D, Mayer F, Farina D. Motor Neuron Pools of Synergistic Thigh Muscles Share Most of Their Synaptic Input. *J Neurosci* 2015;35:12207–12216.
  - [22] Lin F, Wang G, Koh JL, Hendrix RW, Zhang L. In vivo and Noninvasive Three-Dimensional Patellar Tracking Induced by Individual Heads of Quadriceps. *Med. Sci. Sport. Exerc.* 2004;36:93–101.
  - [23] Madeleine P, Leclerc F, Arendt-Nielsen L, Ravier P, Farina D. Experimental muscle pain changes the spatial distribution of upper trapezius muscle activity during sustained contraction. *Clin Neurophysiol* 2006;117:2436–2445. doi:10.1016/j.clinph.2006.06.753.
  - [24] Murray GM, Peck CC. Orofacial pain and jaw muscle activity: a new model. *J. Orofac. Pain* 2007;21:263-278-288.
  - [25] Le Pera D, Graven-Nielsen T, Valeriani M, Oliviero A, Di Lazzaro V, Tonali PA, Arendt-Nielsen L. Inhibition of motor system excitability at cortical and spinal level by tonic muscle pain. *Clin. Neurophysiol.* 2001;112:1633–1641.
  - [26] Salomoni SE, Ejaz A, Laursen AC, Graven-Nielsen T. Variability of three-dimensional forces

- increase during experimental knee pain. *Eur. J. Appl. Physiol.* 2013;113:567–575.
- [27] Schabrun SM, Burns E, Hodges PW. New insight into the time-course of motor and sensory system changes in pain. *PLoS One* 2015;10:1–14.
  - [28] Spencer JD, Hayes KC, Alexander IJ. Knee joint effusion and quadriceps reflex inhibition in man. *Arch Phys Med Rehabil* 1984;65:171–177.
  - [29] Svensson P, Miles TS, McKay D, Ridding MC. Suppression of motor evoked potentials in a hand muscle following prolonged painful stimulation. *Eur. J. Pain* 2003;7:55–62.
  - [30] Tucker K, Hodges PW, Van Den Hoorn W, Nordez A, Hug F. Does stress within a muscle change in response to an acute noxious stimulus? *PLoS One* 2014;9.
  - [31] Tucker K, Larsson AK, Oknelid S, Hodges P. Similar alteration of motor unit recruitment strategies during the anticipation and experience of pain. *Pain* 2012;153:636–643.  
doi:10.1016/j.pain.2011.11.024.
  - [32] Tucker KJ, Hodges PW. Changes in motor unit recruitment strategy during pain alters force direction. *Eur. J. Pain* 2010;14:932–938. doi:10.1016/j.ejpain.2010.03.006.
  - [33] Tucker KJ, Hodges PW. Motoneurone recruitment is altered with pain induced in non-muscular tissue. *Pain* 2009;141:151–155. doi:10.1016/j.pain.2008.10.029.

## FIGURES:

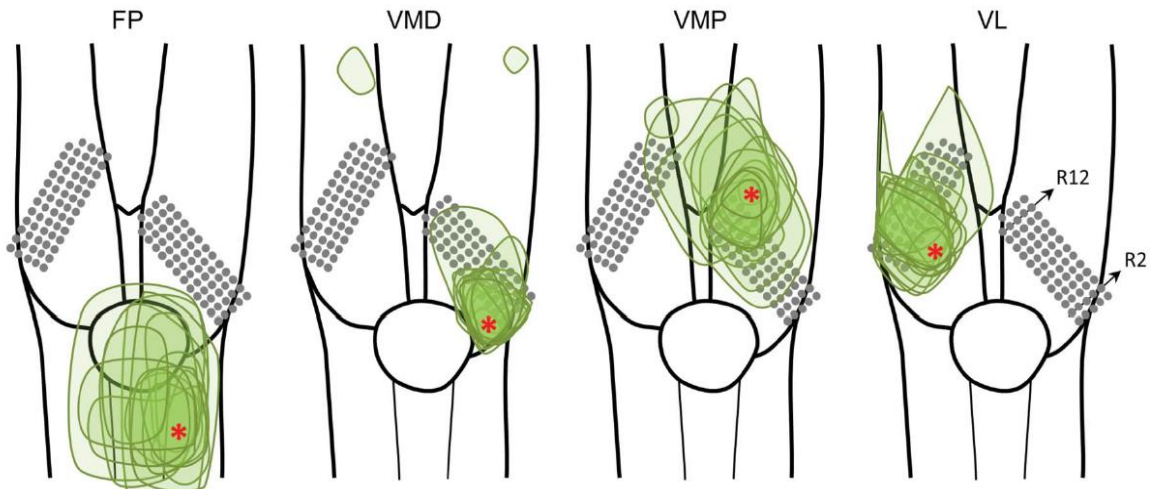


Figure 1: Experimental setup and pain areas for the four locations. Black stars identify the location of the hypertonic saline injections, areas outline by grey lines identify the painful region for each participant. Rows 2 (R2) and 12 (R12; reference for hypertonic saline injection) are identified for the electrode grid placed on the vastus medialis in the far right panel. FP – infrapatellar fat pad; VMD – distal vastus medialis; VMP – proximal vastus medialis; VL – vastus lateralis.

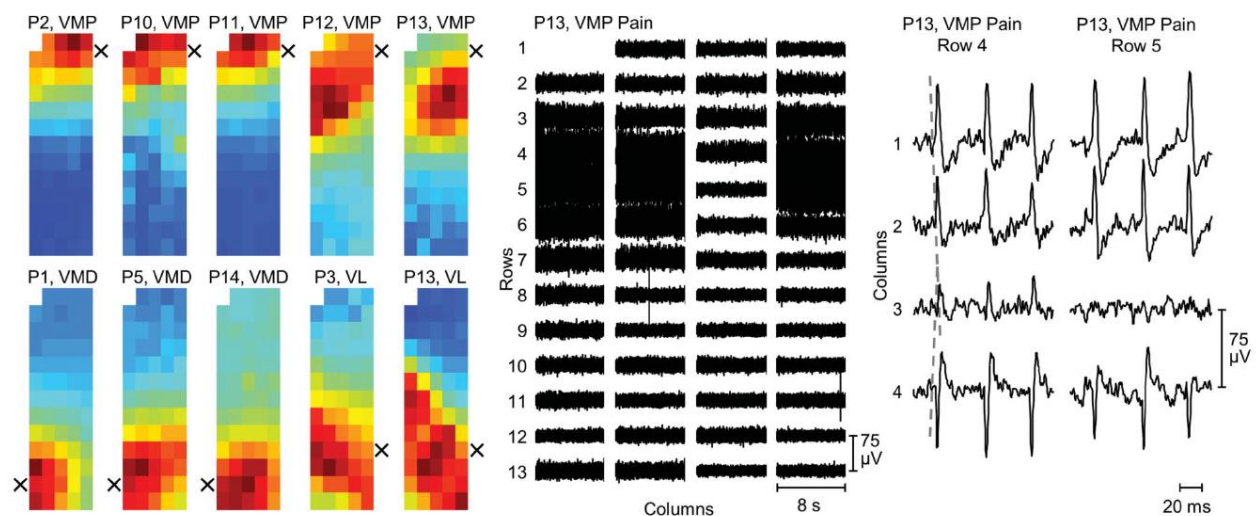


Figure 2: Involuntary muscle activation recorded during pain at rest. Left panels: monopolar average rectified value (ARV) amplitude distributions that illustrate the location of cramps and repetitive muscle twitching of proximal (VMP) and distal (VMD) vastus medialis or vastus lateralis (VL) for individual

participants (P1, P2, etc.). Dark and white boxes identify low and high amplitude values, respectively. Crosses indicate the location of the hypertonic saline injection. Middle panel: Single differential surface EMG signals of a cramp in VMP of one participant after hypertonic saline injection into VMP. Right panel: Details of rows 4 and 5 from same recording as middle panel; the innervation zone (intersection of hashed lines) and action potential propagation (angle of hashed lines) can be observed across columns.

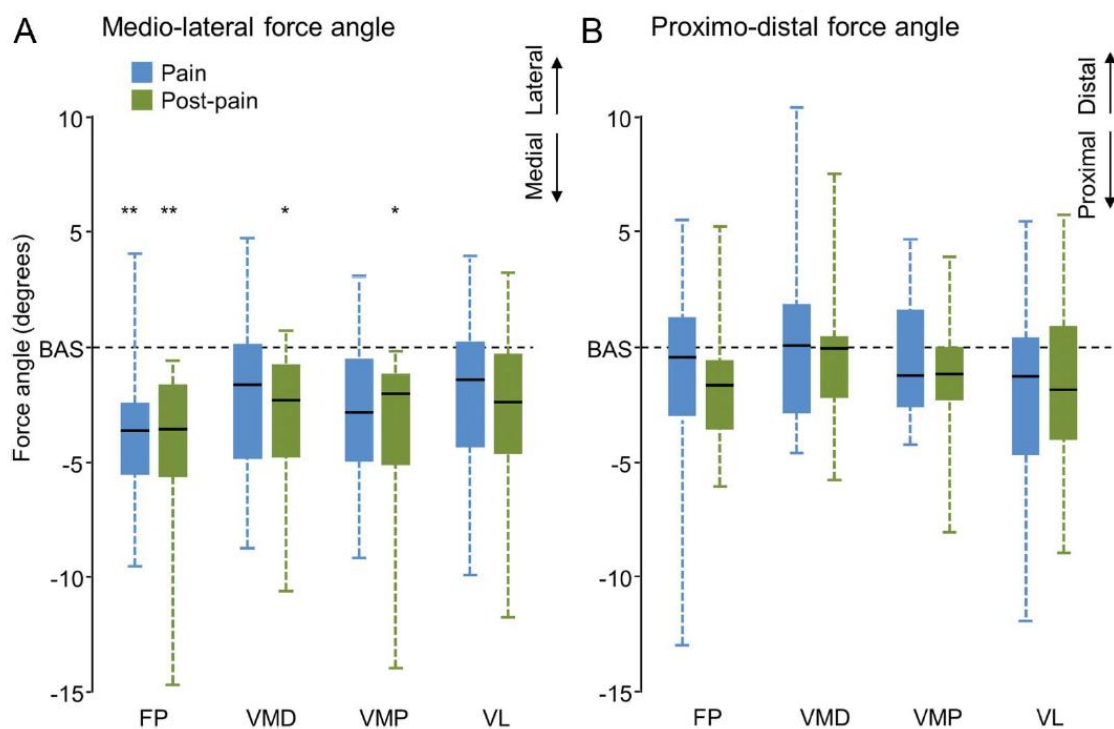


Figure 3: Effect of experimental pain on medio-lateral (A) and proximo-distal (B) knee extension force direction. Boxplots (median, 25<sup>th</sup>-75<sup>th</sup> quartiles, range) show the changes in knee force angle from baseline (BAS) for each hypertonic saline injection location (FP – infrapatellar fat pad; VMD – distal vastus medialis; VMP – proximal vastus medialis; VL – vastus lateralis), during (Pain) and after pain (post-pain). \*\*  $p=0.01$ ; \*  $p<0.05$ .

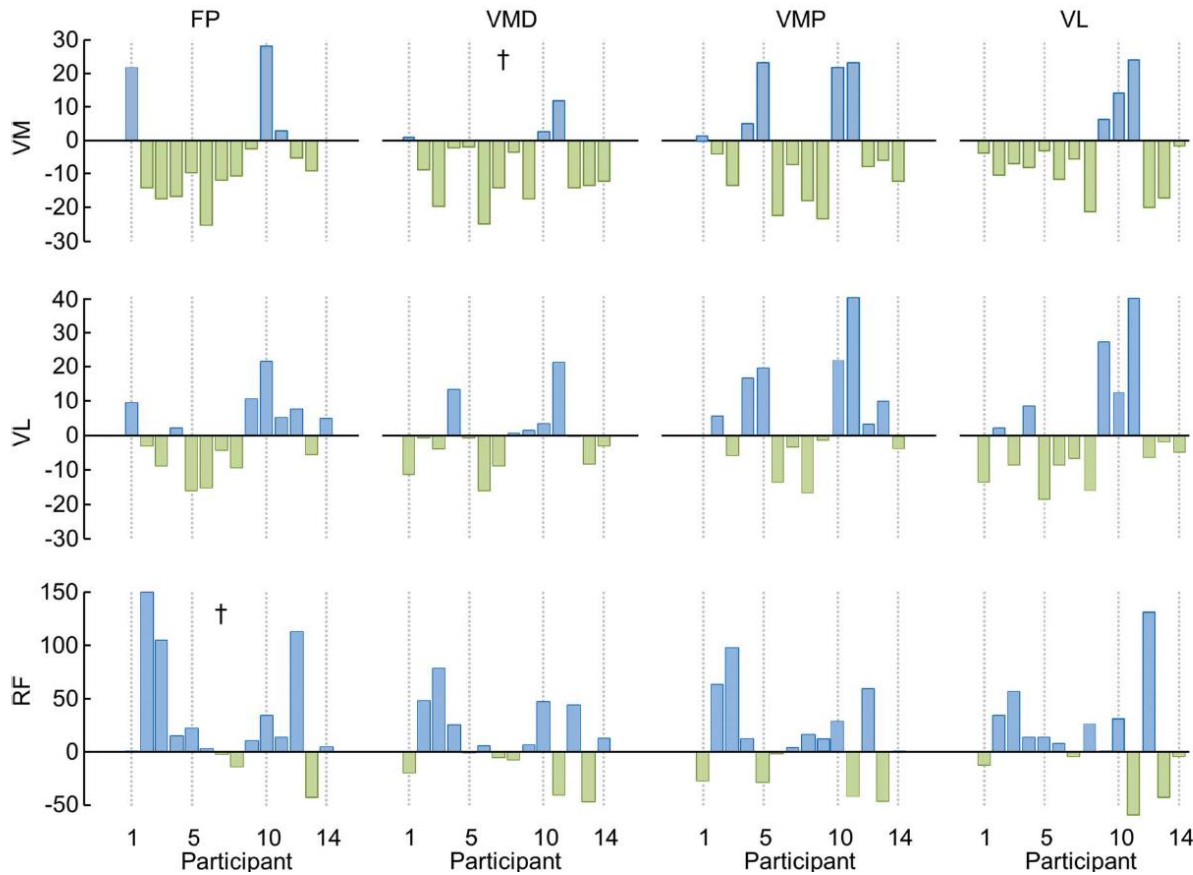


Figure 4: Effect of experimental pain on EMG amplitude during pain for individual participants. For each muscle (VM – vastus medialis; VL – vastus lateralis; rectus femoris - RF), and hypertonic saline injection location (FP – infrapatellar fat pad; VMD – distal vastus medialis; VMP – proximal vastus medialis; VL – vastus lateralis), black and gray bars represent increased and decreased activation, respectively, from baseline for each participant. Changes are shown as percentages of the baseline score. Friedman tests identified main effects for VM and RF. Post-hoc comparisons are displayed, † -  $p < 0.09$

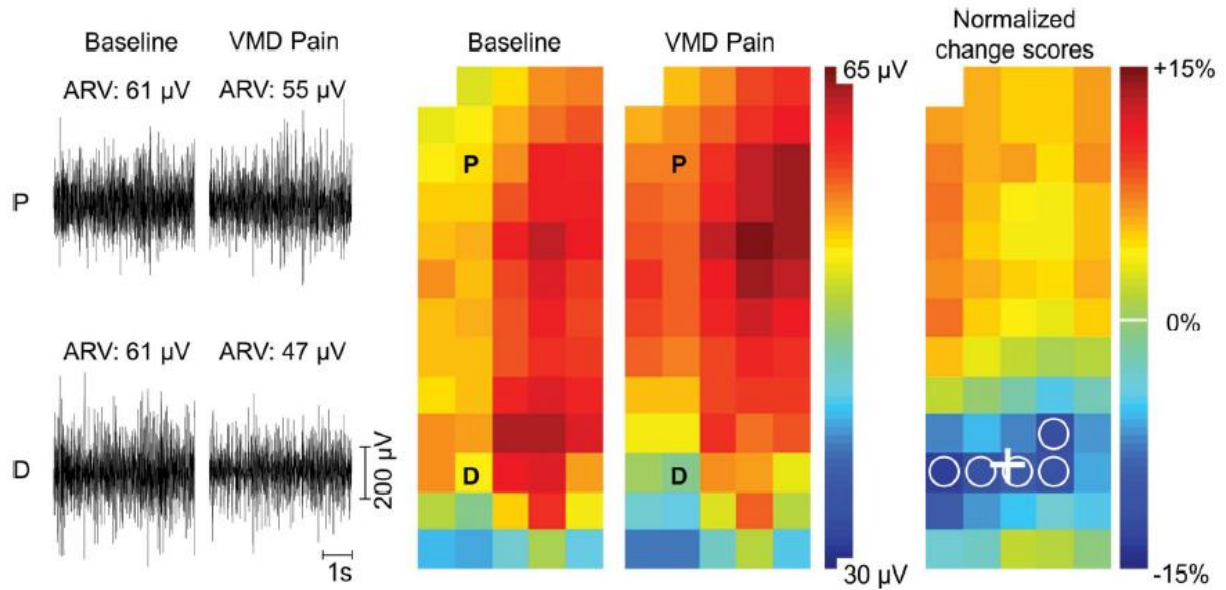


Figure 5: Regional activation in response to experimental pain. Left panel: Surface EMG collected from proximal (P) or distal (D) monopolar channel at baseline or when the distal vastus medialis (VMD) was injected with hypertonic saline. Middle panels: Surface EMG amplitude distribution for the two conditions, averaged over the five contractions; P and D identify channels plotted in the left panel. Right panel: Normalized change scores that describe the amplitude change from baseline to VMD, expressed as a percentage of baseline, for each channel of the electrode grid. White circles identify the five channels with lowest normalized change score, and the white cross identifies their barycenter. ARV – average rectified value.



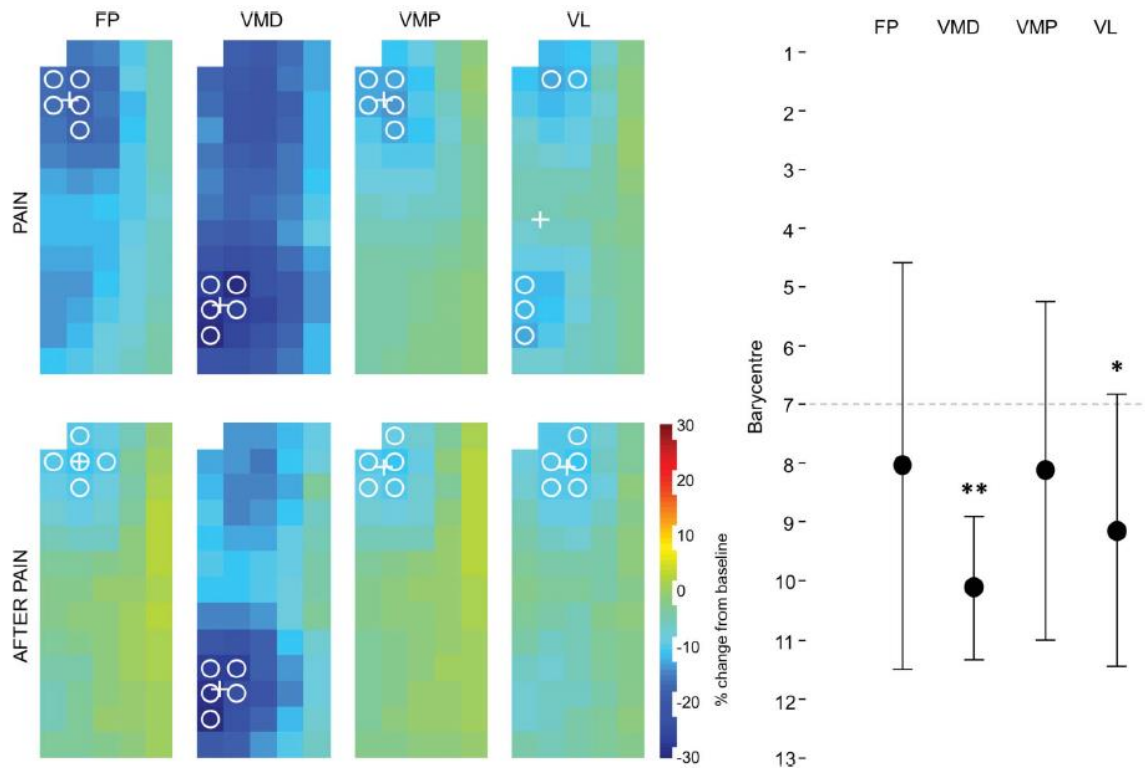


Figure 6: Changes in surface EMG amplitude distribution. Left panels: Normalized change score distributions for the vastus medialis (VM) of a representative participant. White circles identify the channels with lowest change scores, and white crosses indicate their barycenter. Surface EMG amplitude is consistently decreased in the distal region of the VM when distal vastus medialis (VMD), but not proximal vastus medialis (VMP), vastus lateralis (VL) or the infrapatellar fat pad (FP) are injected with hypertonic saline, both during (Pain) and after pain (Post-pain). Right panel: Group mean (standard deviation) position of the barycenter of the channels with lowest normalized change scores. \*\*  $p < 0.001$ , \*  $p < 0.05$ .